Do Weather Conditions Correlate with Findings in Failed, Provision-Filled Nest Cells of *Megachile rotundata* (Hymenoptera: Megachilidae) in Western North America?

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ABSTRACT Cavity-nesting alfalfa leafcutting bees, *Megachile rotundata* (F.) (Hymenoptera: Megachilidae), are excellent pollinators of alfalfa, *Medicago savita* L., for seed production. In commercial settings, artificial cavities are placed in field domiciles for nesting and, thereby, bee populations are sustained for future use. For this study, cells from leafcutting bee nests were collected in late summer from commercial seed fields. Over 3 yr (2003–2005), 39 samples in total of ≈1,000 cells each were taken from several northwestern U.S. states and from Manitoba, Canada. X-radiography of 500 cells from each sample was used to identify "pollen balls" (i.e., cells in which the pollen–nectar provision remained, but the egg or larva, if present, was not detectable on an x-radiograph). Most U.S. samples seemed to have higher proportions of pollen ball cells than Manitoba samples. Pollen ball cells were dissected to determine the moisture condition of the mass provision and true contents of each cell. Most pollen ball cells from Manitoba samples contained fungus, the frequency of which was positively correlated with cool, wet weather. In the United States, most pollen ball cells had moist provisions, and many of them lacked young brood. Correlation analysis revealed that pollen ball cells occurred in greater proportions in fields with more hot days (above 38°C). Broodless pollen ball cells occurred in greater proportions under cool conditions, but dead small larvae (second–third instars) seemed to occur in greater proportions under hot conditions. Pollen ball cells with unhatched eggs and first instars (in the chorion) occurred in lesser proportions under hot conditions.

KEY WORDS Apoidea, Megachilidae, *Medicago*, alfalfa leafcutting bee, pollen ball

The alfalfa leafcutting bee, *Megachile rotundata* (F.) (Hymenoptera: Megachilidae), is a cavity-nesting bee that has been managed for pollination of alfalfa, *Medicago sativa* L., since the 1960s (Stephen 1955, 1962; Bohart 1957, 1972; Stephen and Torchio 1961; Hobbs 1964, 1972; Richards 1984). In alfalfa seed fields, female bees nest in elongate holes in wood or polystyrene boards. They line their nest cavities with pieces of leaves and petals cut from alfalfa or other available plants. For each nest cell, pollen and nectar are gathered for making a mass provision on which an egg is laid and the subsequent developing larva feeds. Through the act of gathering pollen and nectar, female bees incidentally pollinate the alfalfa flowers. Once the bee larva reaches the fifth instar, it spins a cocoon where it remains a prepupa throughout the winter. In the following summer, the prepupa completes development to a pupa and then an adult. Prepupal cells may be artificially incubated after the winter for adult emergence in time for alfalfa bloom (Richards 1984).

According to a 2003–2004 survey (International Seed Federation 2006), 31,000 tons of alfalfa seed was harvested in the United States, and seed production typically is enhanced by the pollination efficiency of commercially available alfalfa leafcutting bees. Alfalfa seed producers using these bees make efforts to sustain bees from year to year, but in the United States, some producers have very poor bee production compared with their Canadian counterparts. The fungus *Ascosphaera aggregata* Skou causes chalkbrood, a fatal disease of alfalfa leafcutting bee larvae (Skou 1975). Canadian producers have been able to keep this disease in check, but in the United States chalkbrood can account for 20% or more of nest cells observed in bee populations at the end of the nesting season (unpublished data). However, the most prevalent loss of alfalfa leafcutting bees in the United States and Canada is attributable to nonproductive cells called "pollen ball" cells, for which there are possibly multiple causes and no known cure (Bohart 1971).

A pollen ball cell is one in which the mass (pollen–nectar) provision was not consumed by a developing larva by the end of the summer. Pollen ball cells vary in their content (Pitts-Singer 2004), and they may contain unhatched eggs or dead small larvae, fungal masses, debris from previous invasions by nest destroying insects that devour larvae, the provision mass, or both (Eves et al. 1980), or lack brood altogether.

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Several plausible explanations have been offered as to why bee brood fails to develop in these nest cells. One suggestion is an inadequate concentration of nectar in the mass provision. Laboratory observations of egg and larval development showed that too much nectar can cause suffocation, and too little nectar can cause starvation and desiccation (Tirgari 1963). Nectar may become a limited resource when the number of foraging bees in a field is very high, in which case nesting female bees may not be able to provide adequate provisions for their brood (Undurraga 1978, Kemp and Bosch 1998, Strickler and Freitas 1999). In commercial alfalfa seed fields, bees may not be released into the field until after the peak of the alfalfa blooming period, and this error in timing may further inhibit the ability of a female bee to find sufficient resources to properly provision nest cells (Strickler and Freitas 1999, Bosch and Kemp 2005).

Another possible explanation for the unconsumed provisions is a late-season onset of senility as adult female bees grow old, leading to improper provisioning of nest cells or their failure to lay an egg (Tirgari 1963). Diseases and toxins also may cause early brood mortality (Goerzen 1991). Most chalkbrood-infected bees die as fully grown larvae, but occasionally, they die as very small larvae (James 2005). One study shows that naturally occurring saponins from alfalfa leaves can be toxic to the bee larvae (Thorp and Briggs 1972), whereas others failed to find such effects (Undurraga 1978, Mayer 1992).

Temperature has been shown to affect early brood (egg through fourth instar) mortality in field and laboratory studies (Eves and Johansen 1974, Tepedino and Parker 1986, Whitfield and Richards 1992). Eggs and larvae do not thrive in the laboratory at temperatures higher than 40°C (Undurraga 1978, James 2005), whereas at the other extreme, immature survival is reduced at a low temperature of 15°C (Tasei and Masure 1978, Whitfield and Richards 1992, James 2005). In fields, cool temperatures at night may inhibit larval feeding and impair further larval development (Hobbs and Richards 1976). Relative humidity also can affect bee development and mortality. Bees reared at low humidity (22%) and across a range of temperatures have slower development rates and higher mortality compared with bees reared at the same temperatures but higher humidity (32, 42, and 55%) (Tirgari 1963). In the field, the negative effects of ambient temperature and humidity on immature bee mortality might be tempered by the appropriate placement of bee domiciles in the field and by providing ventilation and insulation within domiciles (Undurraga 1978, Stephen 1981, Richards 1996).

Here, we investigate whether environmental conditions in the field correlate specifically with the prevalence of pollen ball cells and their various types found at the end of the nesting season in commercial populations of alfalfa leafcutting bees. The first objective was to determine the incidence of pollen ball cells in diverse populations, updating the current status of this problem. The second objective was to determine the range of moisture conditions of the pollen–nectar provisions and categorize cell contents. The final objective was to evaluate the relationship between ambient temperature and humidity with respect to 1) the incidence of pollen ball cells, 2) the moisture condition of these cells, and 3) the content category of these cells.

Materials and Methods

Before releasing their bees in the field, alfalfa seed producers were given HOBO dataloggers (Onset Computer Corp., Bourne, MA). The producers were asked to place the dataloggers in the field domiciles (avoiding direct sunlight and moisture) with the bees to record ambient temperature and relative humidity data throughout the nesting season. For 3 yr (2003–2005), after the nesting season but before alfalfa leafcutting bee cells were removed from nesting boards for winter storage (September–December), cells were obtained from producers. In total, 39 cell samples were taken from fields in Idaho, Oregon, Montana, Nevada, Utah, Washington, Wyoming, and Manitoba, Canada (see Figs. 2–4 for number of samples each year). However, samples were not always taken from each location in each year, and the field sites in each location were not always the same each year. Each sample was a section of a large commercial bee board chosen by the bee manager. The section contained 1,000–1,500 bee cells. At the laboratory, one-cell thick slices of the board containing at least 500 random cells were x-radiographed for visual inspection and evaluation of cells (Stephen and Undurraga 1976). Approximately 500 cells from each sample were scored according to whether they contained live prepupae, dead bees (larvae, prepupae, pupae, or adults), unused provision with no detectable brood (=pollen ball cells), or parasites and insect pests (which were still present or had left evidence of having been present). Identified pollen ball cells from each sample (up to 203 cells) were dissected to record moisture conditions and provide a visual inspection of cell contents.

The moisture condition first was evaluated subjectively by one individual and considered "wet" if the provision was either very soft or liquid (i.e., soupy), "moist" if the provision was firm but pliable (i.e., puttylike), and "dry" if the provision was either hardened or powdery. Upon dissection, some cell contents were found to be completely composed of fungal hyphae or mold (=fungus-filled), although such a cell seemed on x-ray to contain a solid provision-like mass. These fungus-filled cells could not be scored as wet, moist, or dry. The contents of the dissected cells were further divided into seven categories. The categories were 1) broodless, with no other material present on the pollen–nectar mass; 2) an unhatched egg or first instar still within the chorion, often occurring as a collapsed egg or egg imprint; 3) a dead, predefecating larva (second–third instar); 4) a dead, defecating larva (fourth instar); 5) a dead small larva infected with chalkbrood fungus; 6) a cell previously invaded by an insect pest (as evidenced by presence of frass or other debris) and no brood present; and 7) a provision having fun-
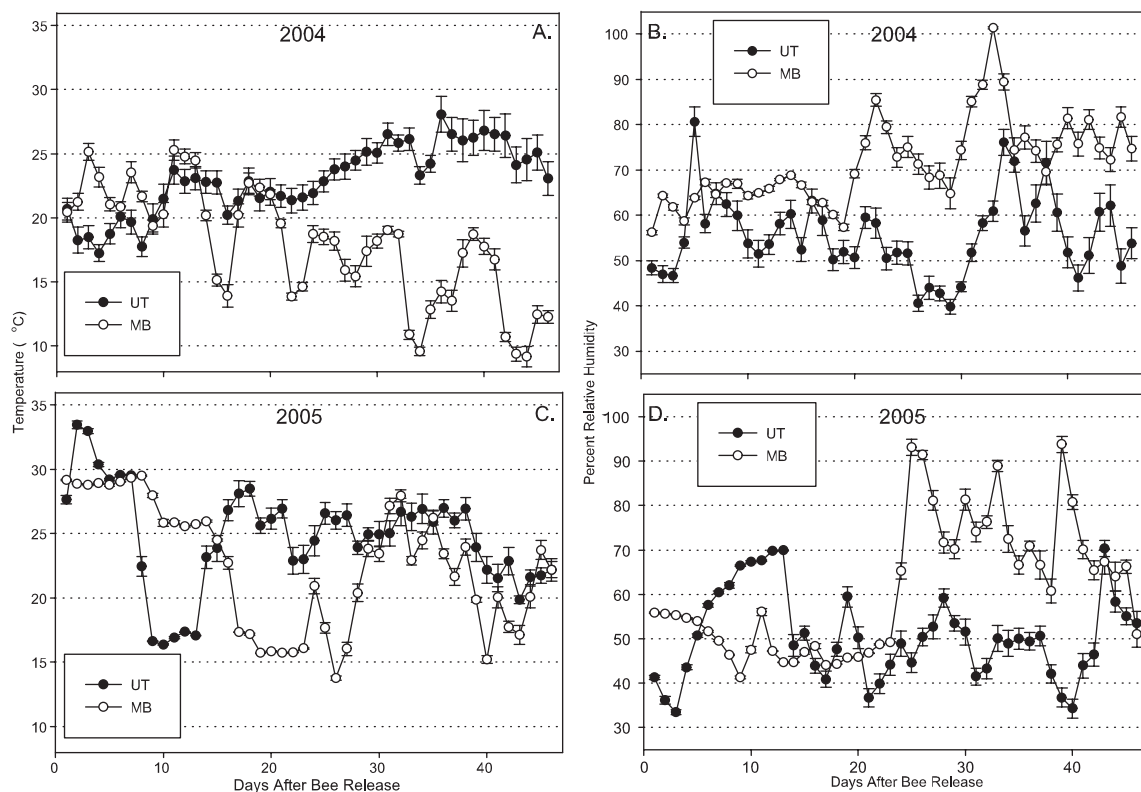
Fig. 1. Daily temperatures and percentage of relative humidity (mean ± SE) recorded in 2004 (A and B) and 2005 (C and D) for one Utah and one Manitoba site.

gus (hyphae or spores) atop a pollen–nectar mass, and no brood present.

Simple Pearson correlation analysis (SAS 2002–2003) was used to evaluate the effect of weather on the incidence of pollen ball calls and their various types. For all 500-cell samples, the proportion of pollen ball cells was calculated and tested for any correlation to the number of days where 1) the mean temperature was >32 or 27°C, or <21, 16, or 10°C; 2) the minimum temperature was <21, 16, or 10°C; 3) the maximum temperature was >38, 32, or 27°C; 4) the mean relative humidity was >100, 90, or 80%; 5) the minimum relative humidity was below 60, 40, 20, or 10%; or 6) the maximum relative humidity was >100 or 90%, or <60, 40, 20, or 10%. Furthermore, the proportion of the identified pollen ball cells (a subset of the original 500-cell sample) having each moisture condition and the proportion of those cells having each content category was tested for any correlation to the same environmental parameters described above. The particular environmental parameters were chosen based on knowledge of *M. rotundata* life history and geographic distribution, records of temperature and humidity extremes found among sample sites, and previous studies of the effects of temperature and humidity on immature bee survival (e.g., Tigari 1963, Tasei and Masure 1978, Undurraga 1978, Whitfield and Richards 1992). Correlation analyses were performed

for each year, and if no significant correlations were found in any year for a particular correlation, all years were pooled together. Environmental data for the analyses included from day 1 (the day when bees were first released in the field) through day 45. Furthermore, significant correlations influenced solely by one or two outlying data points are discussed. Outliers were removed from the data set, and analyses were repeated on remaining samples.

Results

Temperature and Relative Humidity. The mean daily temperature and relative humidity recorded from the Manitoba sites (represented by one of two sites) differed greatly from that of U.S. locations (represented by one Utah site) (Fig. 1). During more than half of the 2004 study period, the Manitoba mean temperatures and humidities were cooler and wetter than those in Utah (Fig. 1A and B). In 2005, the difference in mean temperature between sites was more apparent for about the first two thirds of the study period, with lower temperatures during mid-season in Manitoba (Fig. 1C). The difference in mean relative humidity was again higher in Manitoba compared with Utah in the second half of the study period (Fig. 1D). In Manitoba, the 2004 season was cooler and wetter than the 2005 season.

Distribution of Cells from X-Ray Analysis and Visual Inspections. Some of the 500-bee cell samples had few pollen ball cells, whereas others contained a high proportion (pollen ball cells/sample among all 39 samples from 2003 to 2005: range, 17–203; mean, 84.2; and median, 89). X-radiograph analysis showed that the average percentage of pollen ball cells ranged from 4 to 42% across all samples, and it was lowest in samples from Wyoming (9.2%) in 2003 (Fig. 2A); from Nevada (mean, 7%), Oregon (mean, 9.9%), and Manitoba (mean, 6.2%) in 2004 (Fig. 2B); and from Montana (mean, 8.1%), Washington (mean, 9.9%), and Manitoba (4.2%) in 2005 (Fig. 2C). The samples with the highest average percentage of live prepupae were from Wyoming (68.8%) in 2003 (Fig. 2A), from Manitoba (mean, 87.5%) in 2004 (Fig. 2B), and also from Manitoba (91.8%) in 2005 (Fig. 2C).

For all years, the moist category was the most frequent moisture condition found in the pollen ball samples (Fig. 3). Wet provisions were prevalent in samples from Idaho (2003; mean percentage of pollen ball cells, 53.2%) and Montana (2003: mean, 50.0%; 2004: mean, 70.8%) (Fig. 3A and B). No locality displayed any consistent tendency to have many dry provisions, although samples from Manitoba and Idaho averaged >18% dry provisions in 2005 (Fig. 3C). Many fungus-filled (rather than provision-filled) cells were found in samples from Idaho (mean percentage of pollen ball cells, 23.8% in 2003 and 23.9% in 2005), Oregon (32.3% in 2003 and 44.4% in 2004), and Manitoba (64.5% in 2004) (Fig. 3A and B).

The greatest average percentage of the dissected pollen ball cells that were "broodless" (i.e., no egg or larva detected) was found in samples from Wyoming (mean, 47.1%) in 2003 (Fig. 4A), from Montana (mean, 61.8%), Nevada (mean, 63.0%), Utah (mean, 56.2%), Washington (mean, 61.0%), and Wyoming (mean, 62.7%) in 2004 (Fig. 4B), and from Montana (mean, 67.5%), Nevada (mean, 75.4%), Oregon (mean, 57.3%), and Washington (mean, 61.3%) in 2005 (Fig. 4C). Many of the pollen ball cells contained evidence of an unhatched egg or first instar still inside the egg chorion (Fig. 4), and this result was most prevalent in the 2003 and 2004 Washington samples (mean, 47.0 and 39.0%, respectively) (Fig. 4A and B). Dead predefecating larvae represented the next most abundant category for U.S. samples (mean, <19%). The dissected Manitoba cells had provisions that were mostly moldy (mean, 63.9%) in 2004 or broodless (mean, 50.0%) in 2005 (Fig. 4B and C).

Correlation Analyses of Percentage of Pollen Ball Cells in Sample Populations. Correlation analysis involving the 500-cell samples revealed no significant results for the proportions of pollen ball cells for any year analyzed separately. Analyses of data from all years pooled revealed only one significant result that was unbiased by samples from outlying data points of the 2004 and 2005 Manitoba samples (explained below). This result was a positive correlation found between the occurrence of pollen balls and the number of days with high maximum temperatures >38°C (R = 0.37, P = 0.02). The percentage of pollen balls for the

Manitoba samples in 2004 was 4.4 and 8%, and in 2005 was 4.2%, and these sample sites had no days with maximum temperature >38°C. The percentage of pollen ball cells in U.S. samples ranged from 3.6 to 42.4%, and the number of days at these sites with maximum temperature >38°C ranged from 0 to 38.

Correlation Analyses for Moisture Condition and Cell Contents, Including 2004 Manitoba Samples. Most of the significant correlation results for samples collected in 2004 were influenced entirely by the two samples from Manitoba, which presented obvious outlying data points. (The 2005 Manitoba sample was not an obvious, influential outlier in that year.) Therefore, these 2004 Manitoba data points were excluded from correlation analyses of moisture conditions and cell contents, and the significant results for the remaining U.S. pollen ball cell samples are reported separately (see below). The results biased by the 2004 Manitoba samples are summarized in Tables 1 and 2.

In the dissected cells from the two Manitoba samples, many were either completely filled with fungus or contained fungus atop the remnant provision (Table 1). That so many cells were fungus-filled accounts for the low proportion of cells with assessable moisture conditions (Table 1). For the most part, however, the percentage of moist cells correlated with a cooler environment (Table 1). Low percentages of broodless cells and cells containing dead defecating larvae also correlated with cool and wet conditions in Manitoba (Table 2).

Correlation Analyses of Moisture Condition and Cell Contents, U.S. Samples Only. A single, outlying sample from Idaho in 2003 presented data showing that this site was especially wet. Also, the bee cells from this site contained a comparatively high proportion of fungus-ridden cells and low proportion of the broodless and "unhatched egg or first instar" categories. This single outlier was solely responsible for many significant outcomes of the 2003 analysis. Additionally, some significant outcomes for 2005 were biased solely by data from an outlying Utah sample that had a comparatively high proportion of the unhatched egg or first instar category and extremely low proportion of the "dead predefecating larva" category. Where these samples were solely responsible for significant findings for 2003 and 2005, results are excluded.

Without the data from outliers just mentioned and without data from the 2004 Manitoba samples, the proportion of cells in the remaining U.S. samples with wet, dry or fungus-filled conditions correlated significantly with some of the environmental parameters analyzed in 2003 and 2005, but not in 2004 (Table 3). When the mean temperature was under 21°C, a greater proportion of the cell provisions was wet, and a lesser proportion was dry. The occurrence of fungus-filled cells coincided with very cool summer weather, although such cool days were quite infrequent and occurred in three of the four fields from the 2003 Idaho sample sites, but not in any of the other U.S. sites sampled.

Considering the results for content categories, correlation results for the broodless category were sig-
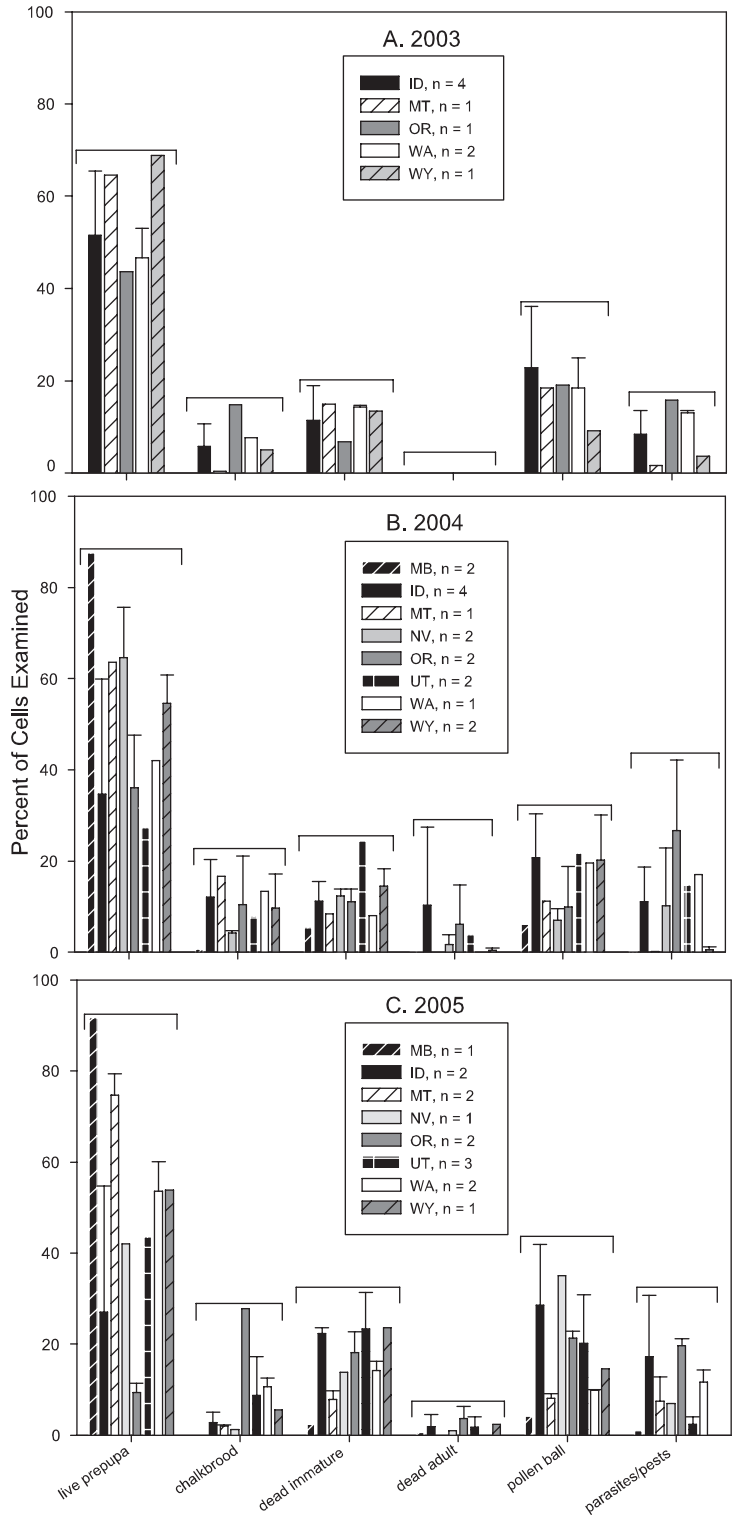
Fig. 2. Results of X-radiograph analysis showing percentage (averaged when *n* >1, error bars are SD) of all types of *M. rotundata* cells from 500-cell samples (*n* is number of samples from each location) from western U.S. states and from Manitoba (MB) in 2003, 2004, and 2005.
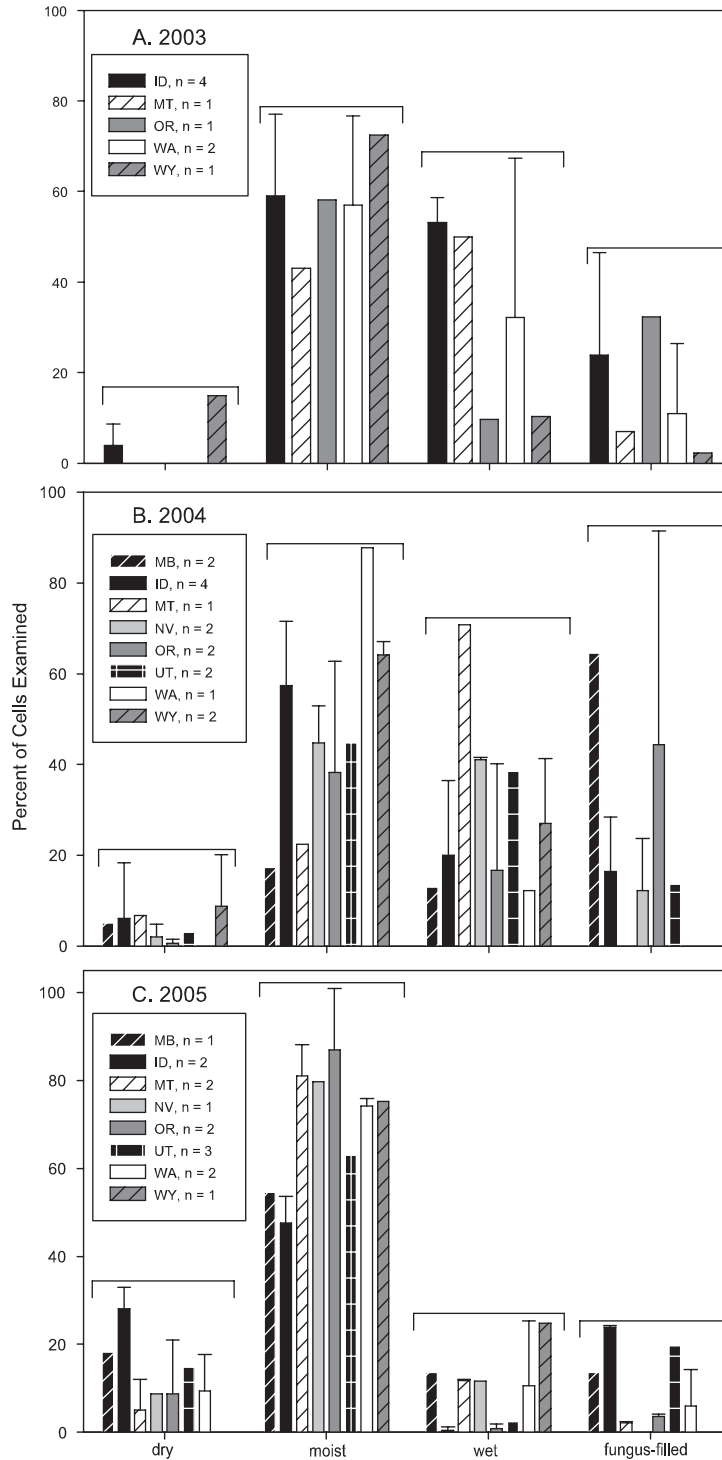
Fig. 3. Results from dissection and inspection of *M. rotundata* pollen ball cells showing percentage (averaged when *n* >1, error bars are standard deviation) of each moisture condition found in samples (*n* is number of samples from each location) from western U.S. states and from Manitoba (MB) in 2003, 2004, and 2005. See text for description of conditions.

nificant only in 2004 (Table 4). The proportion of broodless cells was positively correlated with the number of days with low temperatures. Significant

results showing that a lower proportion of unhatched eggs or first instars occurred with more hot days (32–38°C) are only seen from the 2005 data (Table 4).
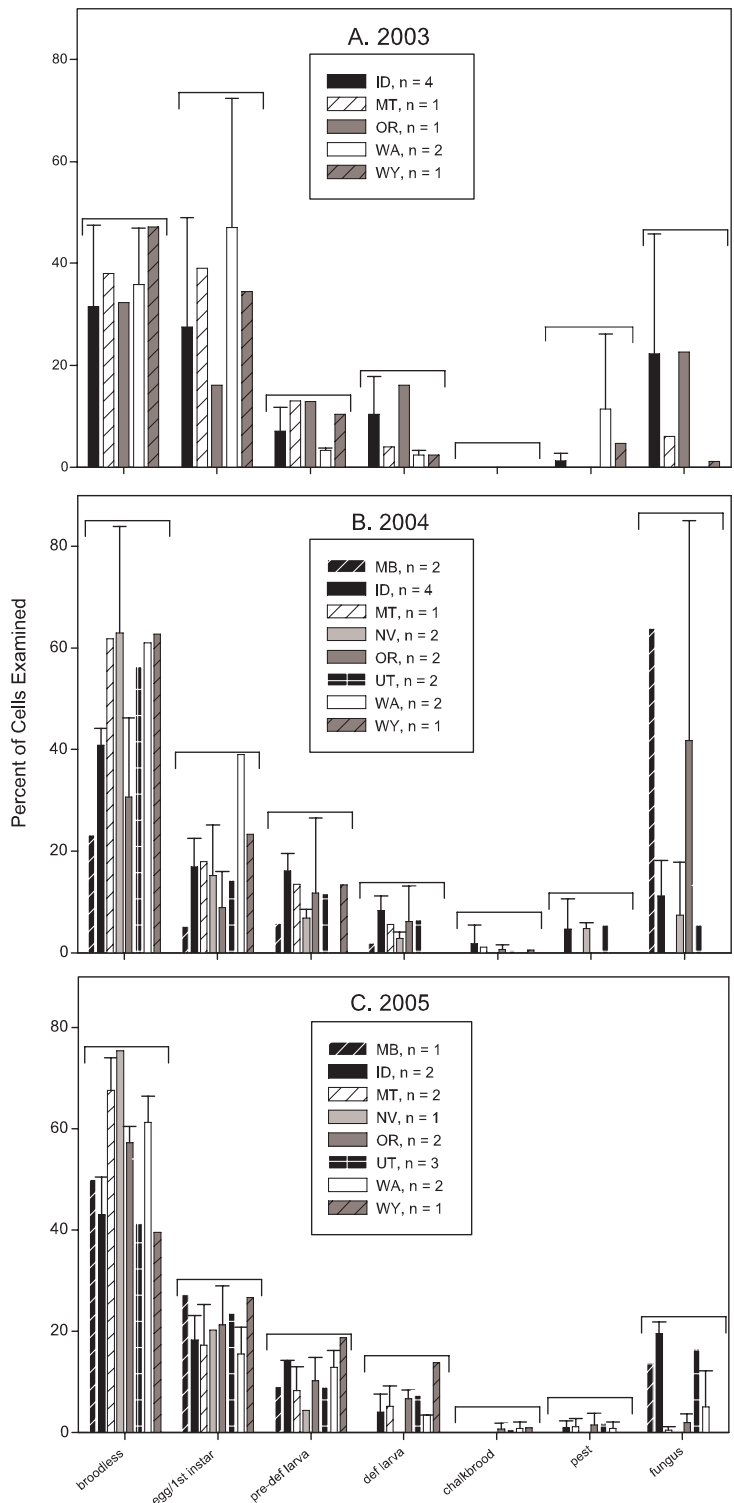
Fig. 4. Results from dissection and inspection of *M. rotundata* pollen ball cells showing percentage (averaged when *n* >1, error bars are SD) of each content category found in samples (*n* is number of samples from each location) from western U.S. states and from Manitoba (MB) in 2003, 2004, and 2005. See text for description of categories. Predef, predefecating; Def, defecating.

Table 1. Percentage of *M. rotundata* pollen ball cells from 2004 Manitoba samples (*n* = 2) having particular moisture conditions and fungus as the content category, and significant results statistics from correlation analysis of all 2004 U.S. and Manitoba (MB) samples with certain environmental parameters (number of days above or below the temperature or humidity condition specified)

Environmental parameters that correlate with each condition or category	No. days parameter met for MB	U.S. + MB correlation statistics (R)	*P* value	Interpretation
Moist condition (14%, 21%)				More cool and wet days correlate with fewer moist provisions. Moist provisions may have been overwhelmed with fungus, as seen below
maxT > 27°C	18, 19	0.61	0.013	
meanT < 10°C	2, 3	0.54	0.027	
meanT < 16°C	15, 15	−0.57	0.020	
maxT < 16°C	2, 2	−0.70	0.002	
maxT < 21°C	11, 13	0.59	0.017	
meanRH < 60%	3, 7	0.56	0.024	
minRH < 40%	7, 12	0.54	0.031	
minRH < 60%	38, 39	0.55	0.026	
meanRH > 80%	7, 8	−0.55	0.027	
meanRH > 90%	1, 2	−0.50	0.048	
Fungus-filled condition (62%, 67%)				More cool and wet days correlate with more cells containing fungus-ridden cells and more fungal growth on provisions
meanT < 10°C	2, 3	0.64	0.007	
meanT < 16°C	15, 15	0.59	0.017	
maxT < 16°C	2, 2	0.59	0.016	
maxT < 21°C	11, 13	0.65	0.006	
maxT > 27°C	18, 19	−0.58	0.020	
minT < 4°C	8, 9	0.55	0.029	
meanRH < 80%	7, 8	0.66	0.005	
meanRH > 90%	1, 2	0.64	0.008	
meanRH < 60%	3, 7	−0.65	0.007	
minRH < 40%	7, 12	−0.66	0.005	
minRH < 60%	38, 39	−0.66	0.005	
Fungus category (61%, 67%)				
meanT < 10°C	2, 3	0.75	0.001	
meanT < 16°C	15, 15	0.73	0.001	
maxT < 16°C	2, 2	0.70	0.002	
maxT < 21°C	11, 13	0.76	0.001	
maxT < 32°C	2, 4	0.58	0.018	
maxT > 27°C	18, 19	−0.70	0.002	
minT < 4°C	8, 9	0.69	0.003	
meanRH < 80%	7, 8	0.79	0.000	
meanRH > 90%	1, 2	0.75	0.001	
meanRH < 60%	3, 7	−0.77	0.001	
minRH < 40%	7, 12	−0.75	0.001	
minRH < 60%	38, 39	−0.77	0.001	

Positive correlations for cells containing provisions with dead predefecating larvae were found when there were more days with relatively low humidity in 2003 (Table 4). The proportion of cells with dead defecating larvae was positively correlated with the number of days with hot weather in 2004 (Table 4). Cells with insect pests or pest debris (and no brood) present were significantly less abundant when there were more days with the maximum relative humidity >90% (Table 4). Other significant correlation results for the "pest" category are not presented because of the bias of an outlying sample from Washington with 22% of this category (compared with other samples with 0–5%). The proportion of cells with fungal hyphae or spores was greater when there were more days with cooler temperatures in 2003 (Table 4), although

Table 2. Percentage of *M. rotundata* pollen ball cells from 2004 Manitoba samples (*n* = 2) having particular content categories, and significant results statistics from correlation analysis of all 2004 U.S. and Manitoba (MB) samples with certain environmental parameters (number of days above or below the temperature or humidity condition specified)

Environmental parameters that correlate with each content category	No. days parameter met for MB	U.S. + MB correlation statistics (R)	*P* value	Interpretation
Broodless category (21%, 26%)				More cool and wet days correlate with fewer cells lacking brood
meanT < 10°C	2, 3	−0.54	0.030	
maxT < 21°C	11, 13	−0.52	0.038	
meanRH < 60%	3, 7	0.52	0.039	
minRH < 40%	7, 12	0.57	0.023	
minRH < 60%	38, 39	0.56	0.024	
meanRH > 80%	7, 8	−0.52	0.038	
meanRH > 90%	1, 2	−0.55	0.026	
Defecating larva category (1%, 3%)				More cool days correlate with very few cells containing dead, defecating larvae
meanT < 21°C	34, 35	−0.51	0.045	

Table 3.   Excluding outlying samples (two from 2004 Manitoba and one from 2003 Idaho), significant correlations of percentage of U.S. *M. rotundata* pollen ball cells having particular moisture conditions with specific environmental parameters (number of days above or below the temperature and relative humidity condition specified)

Environmental parameters (yr) that correlate with each moisture condition (range of %)	Range (d) parameter met	Correlation statistic (R)	P value	Interpretation
Wet (0–25%)				More cool days correlate with more wet provisions
meanT < 21°C (2005)	4–18	0.62	0.018	
Dry (0–15%)				More cool days correlate with fewer dry provisions
meanT < 21°C (2003)	0–10	−0.71	0.050	
Fungus-filled (0–45%)				More cool days correlate with more fungus-ridden cells
maxT < 21°C (2003)	0–3	0.84	0.009	
maxT > 27°C (2003)	41–46	−0.85	0.008	

No significant results were found for the 2004 data. In 2003, $n = 8$; in 2005, $n = 15$.

very few days matched this weather parameter. These results were influenced by the presence of three samples from Idaho (the same samples with the high proportion of cells filled with fungus; Table 3).

Discussion

In all 3 yr of our study, the greatest loss of production (up to 42% of cells) in our survey of *M. rotundata* populations was due to pollen ball cells. The incidence of pollen ball cells was much lower in the Manitoba samples than in most of the U.S. samples. It is logical to surmise that weather plays a major role in the consistently superior ability of Canadian managers to produce and increase bee populations compared with the bee production ability of U.S. managers (Pitts-Singer and James 2005; unpublished 2002–2005 survey data). Indeed, the temperature and humidity data recorded during our study period revealed broad differences between Manitoba and the northwestern United States. It should be noted that changes in relative humidity can be caused not only by natural weather events but also by irrigation.

Studies that address the impact of environmental conditions in the field on brood production fall short of providing definitive causes of the problems (Undurraga 1978, Richards 1996; this study). Furthermore, laboratory findings are not always supported by field observations. However, our exploratory study provides some new, testable hypotheses concerning the condition and contents of failed cells across a large *M. rotundata* production area.

In our physical assessment of the moisture condition of pollen ball cells, we found that almost all were either moist or quite wet (soupy). This observation leads us to believe that the dryness of the provisions provided by the female bee is not a major factor contributing to the loss of brood during early development. Whether the contribution of moisture or nutrients from nectar in the provision meets a critical requirement for the developing larva, or whether the provision changes in its moisture condition as the season progresses, cannot be evaluated from these survey data.

Examination of the contents of pollen ball cells from both the United States and Manitoba revealed that

Table 4.   Excluding outlying samples (two from 2004 Manitoba and one from 2003 Idaho), significant correlations of percentage of U.S. *M. rotundata* pollen ball cells having particular content categories with specific environmental parameters (number of days above or below the temperature and relative humidity condition specified)

Environmental parameters (year) that correlate with each content category (range of %)	Range (d) parameter met	Correlation statistic (R)	P value	Interpretation
Broodless (20–78%)				More cool days correlate with more cells lacking brood
meanT < 21°C (2004)	0–34	0.55	0.043	
minT < 10°C (2004)	0–25	0.58	0.030	
minT < 16°C (2004)	0–44	0.57	0.035	
Egg or first larval stage (12–27%)				More hot days correlate with fewer cells containing unhatched eggs or first instars
maxT > 32°C (2005)	2–41	−0.75	0.001	
maxT > 38°C (2005)	0–26	−0.76	0.001	
Predefecating larva (0–13%)				More dry days correlate with more cells containing dead young larvae
maxRH < 60% (2003)	1–46	0.79	0.018	
Defecating larva (0–11%)				More hot days correlate with more cells containing dead older larvae
maxT > 32°C (2004)	2–43	0.57	0.034	
maxT > 38°C (2004)	0–21	0.54	0.048	
Pest (0–13%)				More wet days correlate with fewer cells invaded by insect pests
maxRH > 90% (2004)	0–30	−0.53	0.037	
Fungal spores/hyphae (0–41%)				More cool days correlate with more cells containing fungus
maxT < 21°C (2003)	1–3	0.97	0.000	

In 2003, $n = 8$; in 2004, $n = 14$; in 2005, $n = 15$.

most were either broodless or contained unhatched eggs or first instars still within the egg's chorion. Dead predefecating larvae (second and third instars) were the third most common finding in the dissected cells. Indeed, Eves and Johansen (1974) examined samples of all dead cells in a population (not just the pollen ball cells) and found that most early brood mortality occurred in the second and third larval stages.

Undurraga (1978) reported that *M. rotundata* bee populations from fields in Oregon and Nevada had as high as 31–63% mortality of eggs and early instars. Although, the provisions were found to be adequate for brood development, the eggs, first, and second instars had either collapsed or the cuticle walls had ruptured. We found no sample from Oregon or Nevada bee populations that contained >8% early brood mortality. However, early brood mortality accounted for 28% of the cells in one sample from Idaho and 15% of cells from a Washington sample in 2003. In 2004 and 2005, we never found >11% early mortality in these same areas. Our broad, multiyear survey reveals that there are annual, as well as regional, differences in mortality of the different life stages.

In Canadian *M. rotundata* populations, larvae are known to be affected by saprophytic molds or fungi (e.g., *Penicillum* sp. and *Aspergillus* sp.), and the fungal growth is influenced by temperature and moisture conditions (Goerzen 1992). These fungi, however, probably are not pathogenic; instead, when conditions are right, they overgrow the nest provisions and possibly starve or poison the developing larvae. Although a very low proportion of pollen ball cells was found in our Manitoba samples, the majority of them were filled with fungal hyphae instead of pollen-nectar masses. Environmental parameters correlating with moldy cells were overwhelmingly influenced by the data points representing the Manitoba samples. The weather conditions that seemed to encourage more mold growth in the Manitoba cells compared with the U.S. cells were a greater number of cool (<16°C) and wet (>80% RH) days. More hot and dry days (>27°C, <40% RH) seemed to discourage the incidence of mold in the Manitoba cells. Generally, lower temperature and higher humidity for the Manitoba samples also may explain the low number of pollen ball cells in 2004.

M. rotundata populations raised in Canada are constituted of a high proportion of live prepupae rather than pollen ball cells at the end of the nesting season, and we correspondingly found few pollen ball cells in our Canadian samples. Therefore, what does this study offer in explaining the pollen ball syndrome in the United States? Hot conditions (>27°C) observed in this study occurred predominantly in the United States, and there was a significant positive correlation between the occurrence of pollen ball cells and hot weather. The occurrence of dead larvae (predefecating and defecating) also increased with the number of dry, hot days. Contrarily, in 2005, the relationship between the number of hot days and the occurrence of unhatched eggs and first instars was negative. The most prevalent cell content category, broodless pollen ball cells, correlated with more cool weather days.

A couple of hypotheses are supported by our data. One is that the survival of eggs and larvae is dependent on meeting certain weather thresholds at particular developmental stages, although the relationship between weather extremes and survival is not exactly clear from our survey data. Another supported hypothesis concerning the prevalence of broodless cells is that female nesting and foraging behavior may be suspended during cool periods causing the interruption of the sequence of nest construction. Furthermore, cool weather (or very hot, dry weather) could inhibit flower bloom or nectar production causing a reduction in resources available for making a proper cell provision in a timely fashion. The interruption in nesting and foraging may cause the female bee to miss the opportunity to lay a mature egg in a newly provisioned cell, and the egg may be reabsorbed by her body. The behavioral repertoire of the nesting bee may then be reset with the capping of the cell instead of laying an egg, followed by the onset of provisioning a new cell. An overall explanation for the various categories and correlating environmental factors may be that the hot weather negatively affects larval development, whereas cool weather affects the nesting behavior of adult females.

Undurraga (1978) reported that *M. rotundata* eggs and young larvae reared in the laboratory at 30°C have >80% survival, but that higher egg mortality occurs if these immature bees are kept at 45°C for 1–3 h. Constant temperature of 50°C will cause 100% larval mortality and this temperature or higher is known to occur in field domiciles (Undurraga 1978). Offspring survival of a related species, *M. apicalis*, was 60% in areas of California where ambient temperatures exceed 50°C (Barthell et al. 1998). However, compared with *M. apicalis*, offspring of *M. rotundata* seem to be less tolerant of such high temperatures. Indeed, Barthell et al. (2002) found higher levels of heat shock protein 70 in *M. rotundata* than in *M. apicalis*, and suggest that the higher levels in *M. rotundata* reflect temperature-induced stress. In our 3-yr study, temperatures recorded in some bee domiciles reached 49°C, and the heat may have been stressful, if not lethal, to some of the immature bees.

At the other end of the spectrum, it has been suggested that extended cool temperatures (15–21°C) may play a role in slow development and early *M. rotundata* mortality (Tigari 1963, Tasei and Masure 1978, Undurraga 1978, Whitfield and Richards 1992). Hobbs and Richards (1976) suggested that late in the nesting season, young larvae may be too cold to feed at low temperatures and fail to complete development. We did not find evidence from our field sampling to support this laboratory study.

Other factors that may contribute to the occurrence of pollen ball cells and to bee reproductive success in general are variations in bee management practices. Such variations include the stocking rates of bees used in the field for seed production, the timing of bee release to assure the availability of floral rewards, the

amount of care given by bee managers to decrease the incidence of disease and parasites, and the interactions of such factors. Another possible cause for pollen balls is an undiagnosed pathogenic disease that causes larval death. These factors that were not addressed by this study could explain why we see so few convincing correlations between the occurrence of pollen ball cells and environmental conditions.

With our broad sampling strategy we are faced with much variability in *M. rotundata* populations and their management. Moreover, with our large number of correlations, we run the risk of finding significant results by chance alone. Thus, our results should remain somewhat speculative. Despite these difficulties, we have still extracted valuable information on the possible effects of temperature and humidity on brood development and *M. rotundata* nesting behavior that can now be tested in more controlled experimental studies. Future studies should also address variations over the nesting season in the types of pollen balls that are produced, rather than looking at these cells at season's end.

Acknowledgments

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